(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 October 2004 (28.10.2004)

PCT

(10) International Publication Number
WO 2004/092332 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number:
PCT/US2004/010729

(22) International Filing Date: 8 April 2004 (08.04.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/461,945	9 April 2003 (09.04.2003)	US
60/462,597	10 April 2003 (10.04.2003)	US
60/462,964	14 April 2003 (14.04.2003)	US
60/462,928	14 April 2003 (14.04.2003)	US
60/463,177	15 April 2003 (15.04.2003)	US
60/463,874	18 April 2003 (18.04.2003)	US
60/470,688	14 May 2003 (14.05.2003)	US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SARS VIRUS POLYPEPTIDES

MSDNGPQSNQRSAPRITFGGPTDSTDNNQNGGRNGARPKQRRPQGLPNNTASW
FTALTQHGKEELRFPRGQGVPINTNSGPDDQIGYYRRATRRVRGGDGKMKELSPR
WYFYYLGTGPEASLPYGANKEGIVWVATEGALNTPKDHIGTRNPNNNAATVLQLPQ
GTTLPKGFYAEGSRGGSQASSRSSSRSRGNSRNSTPGSSRGNSPARMASGGGE
TALALLLLDRLNQLESKVSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKQYNVT
QAFGRRGPEQTQGNFGDQDLIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVT
PSGTWLTYHGAIKLDDKDPQFKDNVILLNKHIDAYKTFPPTEPKKDKKKKTDEAQPL
PQRQKKQPTVTLLPAADMDDFSRQLQNSMSGASADSTQA

(57) Abstract: The present invention provides, *inter alia*, SARS-related polypeptides, *i.e.*, polypeptides associated with Severe Acute Respiratory Syndrome (SARS). The present invention also provides antibodies that specifically bind SARS-related polypeptides. In addition, the present invention provides methods for detecting an infection associated with SARS.

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SARS VIRUS POLYPEPTIDES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of US provisional application nos. 60/461,945, filed April 9, 2003; 60/462,597, filed April 10, 2003; 60/462,964, filed April 14, 2003; 60/462,928, filed April 14, 2003; 60/463,177, filed April 15, 2003; 60/463,874, filed April 18, 2003; and 60/470,688, filed May 14, 2003. Each of the foregoing applications is herein incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] A new disease called severe acute respiratory syndrome (SARS) has been recently reported. The disease was first reported among people in Guangdong Province (China), Hanoi (Vietnam), and Hong Kong (Special Administrative Region of China). It has since spread to other countries. The Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) have received reports of patients with SARS from Canada, China, Hong Kong Special Administrative Region of China, Singapore, Thailand, Vietnam and the United States. To date, more than 100 cases of SARS have been reported in the United States.

[0003] The cause of these illnesses is unknown and is being investigated. Early manifestations in these patients have included influenza-like symptoms such as fever, myalgias, headache, sore throat, dry cough, shortness of breath, or difficulty breathing. In some cases these symptoms are followed by hypoxia, pneumonia, and occasionally acute respiratory distress requiring mechanical ventilation and death. Laboratory findings may include thrombocytopenia and leukopenia.

[0004] It is thought that SARS is spread by close contact between people. In fact, public health experts think that SARS is most likely spread when someone sick with the disease coughs droplets into the air and someone else breaths them in. It is possible that SARS can also be spread more broadly through the air or from touching objects that have been contaminated.

[0005] In view of the foregoing, there is a need to identify the cause of SARS and methods for detecting its causative agent. Quite surprisingly, the present invention fulfills such needs.

SUMMARY OF THE INVENTION

[0006] In one aspect, the present invention provides SARS-related polypeptides. In one embodiment, the present invention provides a SARS-related polypeptide comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18. In another embodiment, the invention provides a SARS-related polypeptide having the amino acid sequence of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18. In yet another embodiment, the present invention provides a SARS-related polypeptide comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:18. In yet another embodiment, the present invention provides a SARS-related polypeptide having the amino acid sequence of SEQ ID NO:1, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:18. In another embodiment, the invention provides a SARS-related polypeptide comprising the amino acid sequence of SEQ ID NO:7. In another embodiment, the invention provides a SARS-related polypeptide having the amino acid sequence of SEQ ID NO:7.

[0007] In another aspect, the present invention provides antibodies that selectively bind SARS-related polypeptides. In one embodiment, the antibody selectively binds a SARS-related polypeptide comprising the amino acid sequence of SEQ ID NO:7, SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18. In another embodiment, the antibody selectively binds a SARS-related polypeptide comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:18.. In another embodiment, the antibody selectively binds a SARS-related polypeptide having the amino acid sequence of SEQ ID NO:7, SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18.

[0008] In another aspect, the present invention provides methods for detecting SARS-associated infections. In one embodiment, the present invention provides a method for

detecting an infection associated with SARS in a sample, the method comprising detecting the presence of a SARS-related polypeptide in the sample, wherein the presence of the polypeptide is indicative of the presence of the SARS-associated infection. In another embodiment, the present invention provides a method for detecting the presence of an infection associated with SARS in a sample, the method comprising: detecting the presence of a SARS-related antibody in the sample, wherein the presence of said SARS antibody is indicative of the presence of the infection. Such methods can be immunodiagnostic assays, immunoblot assays, western blot assays, enzyme linked immunoabsorbent assays (ELISA), *etc.* Alternatively, Surface-Enhanced Laser Desorption Ionization (SELDI) can be used to detect either the SARS-related polypeptides or the SARS-related antibodies and, in turn, the infection associated with the SARS virus.

[0009] In another aspect, the present invention provides kits for detecting antibodies indicative of infection associated with SARS in a mammal. In a preferred embodiment, the kits contain components suitable for detecting antibodies. In a more preferred embodiment, such kits are of the type that can be employed in diagnostic laboratories for detecting antibodies associated with a coronavirus and/or SARS. Possible examples of kits of this type include, but are not limited to, Western blot strips or ELISA plates that are already coated with the polypeptides of the present invention. In addition, the kits of the present invention can comprise other components required to carry out the assay or test, such as the detection component used to detect the polypeptide-specific antibody complex.

[0010] In one embodiment, the detection component for detecting the polypeptide-specific antibody complex is an anti-antibody or another antibody which is directed against a polupeptide of the present invention. This detection component can be labeled with, for example, an enzyme that catalyzes a color reaction (for example, a peroxidase). However, it is also possible for the polypeptide to be labeled. For instance, the polypeptide can be recombinantly produced as a fusion protein, the fusion portion making labeling or removal readily possible.

[0011] Other features, objects and advantages of the invention and its preferred embodiments will become apparent from the detailed description and examples that follow.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Figure 1 comprises the peptides confirmed via MS-MS that are present in the 46kDa protein translated from the SARS genome reading frame 1 (SEQ ID NO:22). Matches are in bold italics and detailed in Example 2.

[0013] Figure 2 illustrates the results obtained on a Ciphergen CM10 chip, which indicate that defensin levels are decreased in individuals with SARS. Black lanes are protein profiles of nasal pharyngeal swabs from metapneumovirus positive patients; Blue (light colored) lanes are protein profiles of nasal pharyngeal swabs from SARS suspected patients.

[0014] Figure 3 illustrates the results obtained on a Ciphergen C18 SEND chip, which indicate that defensin levels are decreased in individuals with SARS..

[0015] Figure 4 illustrates the results obtained using an SELDI immunoassay for detecting defensin levels, which indicate that defensin levels are decreased in individuals with SARS.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

A. DEFINITIONS

[0016] "Biological sample" or "sample," as used herein, refers to a sample of biological tissue or fluid that contains SARS-related polypeptides or that is suspected of containing SARS-related polypeptides, or antibodies to such peptides. Such samples include blood, samples from the respiratory tract, *e.g.*, sputum, throat swabs, nasal samples, *etc.* A biological sample is typically obtained from a eukaryotic organism, most preferably a mammal such as a primate *e.g.*, chimpanzee or human; cow; dog; cat; a rodent, *e.g.*, guinea pig, rat, mouse; rabbit; or a bird; reptile; or fish.

[0017] A "nasopharyngeal sample" refers to any sample from the back of the throat.

[0018] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers.

[0019] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0020] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0021] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

[0022] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0023] The following eight groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
7) Serine (S), Threonine (T); and
8) Cysteine (C), Methionine (M)

[0024] "Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

[0025] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[0026] Antibodies exist, *e.g.*, as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to

produce F(ab)'$_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The F(ab)'$_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)'$_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (*see, Fundamental Immunology* (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (*e.g.*, single chain Fv) or those identified using phage display libraries (*see, e.g.*, McCafferty *et al.*, *Nature, 348*:552-554 (1990)). The term antibody also includes bivalent or bispecific molecules, diabodies, triabodies, and tetrabodies. Bivalent and bispecific molecules are described in *e.g.*, in Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA, 90*:6444-6448 (1993); WO 9311161; EP 404,097; Kostelny *et al,. J. Immunol., 148*:1547 (1992); Gruber *et al.*, *J. Immunol., 152*:5368 (1994); Pack and Pluckthun, *Biochemistry, 31*:1579 (1992); Zhu *et al.*, *Protein Sci., 6*:781 (1997); and McCartney *et al.*, *Protein Eng., 8*:301 (1995).

[0027] For preparation of monoclonal or polyclonal antibodies, any technique known in the art can be used (*see, e.g.*, Kohler & Milstein, *Nature, 256*:495-497 (1975); Kozbor *et al.*, *Immunology Today, 4*:72 (1983); Cole *et al.*, pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy* (1985)) and are further described below. Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies. Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (*see, e.g.*, McCafferty *et al.*, *Nature, 348*:552-554 (1990); Marks *et al.*, *Biotechnology, 10*:779-783 (1992)).

[0028] An "anti-SARS-related peptide" antibody is an antibody or antibody fragment that specifically binds to a polypeptide having the sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5.

[0029] "Defensins," as used herein, are well known in the art and are anti-bacterial or anti-fungal agents (see, U.S. Patent No. 5,242,902, which issued to Murphy *et al.*). Defensins have been found and characterized in many animals, including humans, guinea pig, rat, rabbit, macaques, and mice, as well as in plants and insects. For the purposes

of the present invention, there are two structural classes of mammalian defensins, α and β. The term "defensin," as used in this application, includes both α and β defensins, and in particular α-defensins-1, 2, and 3 (such as human alpha-defensins 1, 2 and 3). Defensins and methods for their detection are disclosed in U.S. Provisional Patent Application No. 60/412414, filed on 09/20/2002, the teachings of which are incorporated herein by reference.

[0030] As used herein, the term "human alpha-defensin" refers to a polypeptide having one of the following amino acid sequences, allelic variants thereof, and pre-proproteins thereof. The sequences for human alpha-defensins 1-6 are as follows; Alpha-defensin 1 (HNP1): ACYCRIPACIAGERRYGTCIYQGRLWAFCC; Alpha-defensin 2 (HNP2): CYCRIPACIAGERRYGTCIYQGRLWAFCC; Alpha-defensin 3 (HNP3): DCYCRIPACIAGERRYGTCIYQGRLWAFCC; Alpha-defensin 4 (HNP4): VCSCRLVFCRRTELRVGNCLIGGVSFTYCCTRV; Alpha-defensin 5 (HD5): ARATCYCRTGRCATRESLSGVCEISGRLY RLCCR; and Alpha-defensin 6 (HD6): TRAFTCHCRRSCYSTEYSYGTCT VM GINHRFCCL. Alpha-defensins are described in United States Patent No. 4,705,777 (Lehrer *et al.*).

[0031] The term "immunoassay" is an assay that uses an antibody to specifically bind an antigen. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the antigen.

[0032] The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5 can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5, and not with other proteins, except for polymorphic variants or alleles of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5. This selection may be achieved by subtracting out antibodies that cross-react with other protein molecules. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays

are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g.*, Harlow & Lane, *Antibodies, A Laboratory Manual* (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

[0033] The phrase "selectively associates with" refers to the ability of an antibody to "selectively (or specifically) bind" to a SARS-realted peptide, as defined above.

[0034] "Mass spectrometer" refers to a gas phase ion spectrometer that measures a parameter that can be translated into mass-to-charge ratios of gas phase ions. Mass spectrometers generally include an ion source and a mass analyzer. Examples of mass spectrometers are time-of-flight, magnetic sector, quadrupole filter, ion trap, ion cyclotron resonance, electrostatic sector analyzer and hybrids of these. "Mass spectrometry" refers to the use of a mass spectrometer to detect gas phase ions.

[0035] "Solid support" refers to a solid material which can be derivatized with, or otherwise attached to, a capture reagent. Exemplary solid supports include probes, microtiter plates and chromatographic resins.

[0036] "Surface-enhanced laser desorption/ionization" or "SELDI" refers to a method of desorption/ionization gas phase ion spectrometry (*e.g.*, mass spectrometry) in which the analyte is captured on the surface of a SELDI probe that engages the probe interface of the gas phase ion spectrometer. In "SELDI MS," the gas phase ion spectrometer is a mass spectrometer. SELDI technology is described in, *e.g.*, U.S. Patent No. 5,719,060 (Hutchens and Yip) and U.S. Patent No. 6,225,047 (Hutchens and Yip).

[0037] "Surface-Enhanced Affinity Capture" or "SEAC" is a version of SELDI that involves the use of probes comprising an absorbent surface (a "SEAC probe"). "Adsorbent surface" refers to a surface to which is bound an adsorbent (also called a "capture reagent" or an "affinity reagent"). An adsorbent is any material capable of binding an analyte (*e.g.*, a target polypeptide or nucleic acid). "Chromatographic adsorbent" refers to a material typically used in chromatography. Chromatographic adsorbents include, for example, ion exchange materials, metal chelators (*e.g.*, nitriloacetic acid or iminodiacetic acid), immobilized metal chelates, hydrophobic interaction adsorbents, hydrophilic interaction adsorbents, dyes, simple biomolecules (*e.g.*, nucleotides, amino acids, simple sugars and fatty acids) and mixed mode adsorbents (*e.g.*, hydrophobic attraction/electrostatic repulsion adsorbents). "Biospecific adsorbent" refers an adsorbent comprising a biomolecule, *e.g.*, a nucleic acid molecule (*e.g.*, an aptamer), a polypeptide, a polysaccharide, a lipid, a steroid or

a conjugate of these (*e.g.*, a glycoprotein, a lipoprotein, a glycolipid, a nucleic acid (*e.g.*, DNA)-protein conjugate). In certain instances the biospecific adsorbent can be a macromolecular structure such as a multiprotein complex, a biological membrane or a virus. Examples of biospecific adsorbents are antibodies, receptor proteins and nucleic acids. Biospecific adsorbents typically have higher specificity for a target analyte than chromatographic adsorbents. Further examples of adsorbents for use in SELDI can be found in U.S. Patent 6,225,047 (Hutchens and Yip, "Use of retentate chromatography to generate difference maps," May 1, 2001).

[0038] "Adsorption" refers to detectable non-covalent binding of an analyte to an adsorbent or capture reagent.

[0039] "Surface-Enhanced Neat Desorption" or "SEND" is a version of SELDI that involves the use of probes comprising energy absorbing molecules chemically bound to the probe surface. ("SEND probe.") "Energy absorbing molecules" ("EAM") refer to molecules that are capable of absorbing energy from a laser desorption/ ionization source and thereafter contributing to desorption and ionization of analyte molecules in contact therewith. The phrase includes molecules used in MALDI , frequently referred to as "matrix", and explicitly includes cinnamic acid derivatives, sinapinic acid ("SPA"), cyano-hydroxy-cinnamic acid ("CHCA") and dihydroxybenzoic acid, ferulic acid, hydroxyacetophenone derivatives, as well as others. It also includes EAMs used in SELDI. SEND is further described in United States Patent No. 5,719,060 and United States patent application 60/408,255, filed September 4, 2002 (Kitagawa, "Monomers And Polymers Having Energy Absorbing Moieties Of Use In Desorption/Ionization Of Analytes").

[0040] "Surface-Enhanced Photolabile Attachment and Release" or "SEPAR" is a version of SELDI that involves the use of probes having moieties attached to the surface that can covalently bind an analyte, and then release the analyte through breaking a photolabile bond in the moiety after exposure to light, *e.g.*, laser light. SEPAR is further described in United States Patent No. 5,719,060.

[0041] "Eluant" or "wash solution" refers to an agent, typically a solution, which is used to affect or modify adsorption of an analyte to an adsorbent surface and/or remove unbound materials from the surface. The elution characteristics of an eluant can depend, for example, on pH, ionic strength, hydrophobicity, degree of chaotropism, detergent strength and temperature.

[0042] "Analyte" refers to any component of a sample that is desired to be detected. The term can refer to a single component or a plurality of components in the sample.

[0043] "Biochip" refers to a solid substrate having a generally planar surface to which an adsorbent is attached. Frequently, the surface of the biochip comprises a plurality of addressable locations, each of which location has the adsorbent bound there. Biochips can be adapted to engage a probe interface and, therefore, function as probes.

[0044] "Protein biochip" refers to a biochip adapted for the capture of polypeptides. Many protein biochips are described in the art. These include, for example, protein biochips produced by Ciphergen Biosystems (Fremont, CA), Packard BioScience Company (Meriden CT), Zyomyx (Hayward, CA) and Phylos (Lexington, MA). Examples of such protein biochips are described in the following patents or patent applications: U.S. Patent No. 6,225,047 (Hutchens and Yip, "Use of retentate chromatography to generate difference maps," May 1, 2001); International publication WO 99/51773 (Kuimelis and Wagner, "Addressable protein arrays," October 14, 1999); U.S. Patent No. 6,329,209 (Wagner *et al.*, "Arrays of protein-capture agents and methods of use thereof," December 11, 2001) and International publication WO 00/56934 (Englert *et al.*, "Continuous porous matrix arrays," September 28, 2000).

[0045] Protein biochips produced by Ciphergen Biosystems comprise surfaces having chromatographic or biospecific adsorbents attached thereto at addressable locations. Ciphergen ProteinChip® arrays include NP20, H4, H50, SAX-2, WCX-2, CM-10, IMAC-3, IMAC-30, LSAX-30, LWCX-30, IMAC-40, PS-10, PS-20 and PG-20. These protein biochips comprise an aluminum substrate in the form of a strip. The surface of the strip is coated with silicon dioxide. In the case of the NP-20 biochip, silicon oxide functions as a hydrophilic adsorbent to capture hydrophilic proteins.

[0046] H4, H50, SAX-2, WCX-2, CM-10, IMAC-3, IMAC-30, PS-10 and PS-20 biochips further comprise a functionalized, cross-linked polymer in the form of a hydrogel physically attached to the surface of the biochip or covalently attached through a silane to the surface of the biochip. The WCX-2 and CM-10 biochips have carboxylate functionalities for cation exchange. The IMAC-3 and IMAC-30 biochips have nitriloacetic acid functionalities that adsorb transition metal ions, such as Cu++ and Ni++, by chelation. These immobilized metal ions allow adsorption of peptide and proteins by coordinate bonding. The PS-10 biochip has carboimidizole functional groups that can react with groups on proteins for covalent binding. The PS-20 biochip has epoxide functional groups for covalent binding with

proteins. The PS-series biochips are useful for binding biospecific adsorbents, such as antibodies, receptors, lectins, heparin, Protein A, biotin/streptavidin and the like, to chip surfaces where they function to specifically capture analytes from a sample. The PG-20 biochip is a PS-20 chip to which Protein G is attached. The LSAX-30 (anion exchange), LWCX-30 (cation exchange) and IMAC-40 (metal chelate) biochips have functionalized latex beads on their surfaces. Such biochips are further described in: WO 00/66265 (Rich *et al.*, "Probes for a Gas Phase Ion Spectrometer," November 9, 2000); WO 00/67293 (Beecher *et al.*, "Sample Holder with Hydrophobic Coating for Gas Phase Mass Spectrometer," November 9, 2000); U.S. patent application US20030032043A1 (Pohl and Papanu, "Latex Based Adsorbent Chip," July 16, 2002) and U.S. patent application 60/350,110 (Um *et al.*, "Hydrophobic Surface Chip," November 8, 2001).

B. SARS-RELATED POLYPEPTIDES AND THEIR PREPARATION

[0047] In one embodiment, the present invention provides a SARS-related polypeptide comprising the amino acid sequence of SEQ ID NO:7, SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18. In another embodiment, the invention provides a SARS-related polypeptide having the amino acid sequence of SEQ ID NO:7, SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:187. In yet another embodiment, the present invention provides a SARS-related polypeptide comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:18. In yet another embodiment, the present invention provides a SARS-related polypeptide having the amino acid sequence of SEQ ID NO:1, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:18.

[0048] The SARS-related polypeptides of the invention can be prepared in a wide variety of ways. For the preferred relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. (See, for example, Stewart & Young, SOLID PHASE PEPTIDE SYNTHESIS, 2D. ED., Pierce Chemical Co., 1984).

[0049] Alternatively, recombinant DNA technology can be employed wherein a nucleotide sequence which encodes an SARS-related polypeptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art, as described in basic texts Sambrook & Russell, *Molecular Cloning, A Laboratory Manual* (3rd Ed, 2001); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., 1994-1999).

[0050] To obtain high level expression of a sequence encoding the SARS-related polypeptides of the invention, a nucleic acid sequence encoding the polypeptide is cloned into an expression vector. Bacterial expression systems for expressing the polypeptide are available in, *e.g.*, *E. coli, Bacillus sp.*, and *Salmonella* (Palva *et al.*, *Gene, 22*:229-235 (1983); Mosbach *et al.*, *Nature, 302*:543-545 (1983). Kits for such expression systems are commercially available. Eukaryotic expression systems for mammalian cells, yeast, and insect cells are also well known in the art and are also commercially available.

[0051] The expressed SARS-related proteins are purified in accordance with known techniques.

C. PREPARATION AND USE OF ANTIBODIES TO SARS-RELATED POLYPEPTIDES

[0052] Antibodies specific to SARS-related peptide are produced using known techniques. A general overview of the applicable technology can be found in Harlow & Lane, *Antibodies: A Laboratory Manual* (1988) and Harlow & Lane, *Using Antibodies* (1999). Methods of producing polyclonal and monoclonal antibodies that react specifically with SARS-related peptide are known to those of skill in the art (*see, e.g.*, Coligan, *Current Protocols in Immunology* (1991); Harlow & Lane, *supra*; Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed. 1986); and Kohler & Milstein, *Nature, 256*:495-497 (1975). Such techniques include antibody preparation by selection of antibodies from libraries of recombinant antibodies in phage or similar vectors, as well as preparation of polyclonal and monoclonal antibodies by immunizing rabbits or mice (*see, e.g.*, Huse *et al.*, *Science, 246*:1275-1281 (1989); Ward *et al.*, *Nature, 341*:544-546 (1989)). Such antibodies can be used for diagnostic applications, *e.g.*, to diagnose SARS, or to evaluate the efficacy of a SARS vaccine..

[0053] Often, a synthetic polypeptide having the sequence of the SARS-related polypeptides described herein, *e.g.*, SEQ ID NO:7, SEQ ID NO:1, SEQ ID NO:6,

SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18 and conjugated to a carrier protein can be used as an immunogen. The product is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies may be generated, for subsequent use in immunoassays to measure the protein.

[0054] Typically, polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against non-non coronavirus proteins or even other related proteins from other coronaviruses using a competitive binding immunoassay. Specific polyclonal antisera and monoclonal antibodies will usually bind with a K_d of at least about 0.1 mM, more usually at least about 1 μM, optionally at least about 0.1 μM or better, and optionally 0.01 μM or better.

[0055] Once specific antibodies are available, binding interactions the peptide can be detected by a variety of immunoassay methods. For a review of immunological and immunoassay procedures, see *Basic and Clinical Immunology* (Stites & Terr eds., 7th ed. 1991) and *Methods in Cell Biology: Antibodies in Cell Biology*, volume 37 (Asai, ed. 1993). Moreover, the immunoassays of the present invention can be performed in any of several configurations, which are reviewed extensively in *Enzyme Immunoassay* (Maggio, ed., 1980); and Harlow & Lane, *supra*.

[0056] Immunoassays also often use a labeling agent to specifically bind to and label the complex formed by the antibody and antigen. The labeling agent may itself be one of the moieties comprising the antibody/antigen complex. Thus, the labeling agent may be a labeled SARS-related polypeptide or a labeled anti-SARS-related polypeptide antibody. Alternatively, the labeling agent may be a third moiety, such as a secondary antibody, that specifically binds to the antibody/ SARS-related polypeptide complex (a secondary antibody is typically specific to antibodies of the species from which the first antibody is derived). Other proteins capable of specifically binding immunoglobulin constant regions, such as protein A or protein G may also be used as the labeling agent. These proteins exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species (*see, e.g.*, Kronval *et al.*, *J. Immunol.*, *111*:1401-1406 (1973); Akerstrom *et al.*, *J. Immunol.*, *135*:2589-2542 (1985)). The labeling agent can be modified with a detectable moiety, such as biotin, to which another molecule can specifically bind, such as streptavidin. A variety of detectable moieties are well known to those skilled in the art.

[0057] Commonly used assays include noncompetitive assays, *e.g.*, sandwich assays, and competitive assays. In competitive assays, the amount of SARS-related peptide present in the sample is measured indirectly by measuring the amount of a known, added (exogenous) peptide displaced (competed away) from an anti-SARS-related peptide antibody by the unknown SARS-related peptide present in a sample. Commonly used assay formats include immunoblots, which are used to detect and quantify the presence of protein in a sample. Other assay formats include liposome immunoassays (LIA), which use liposomes designed to bind specific molecules (*e.g.*, antibodies) and release encapsulated reagents or markers. The released chemicals are then detected according to standard techniques (*see,* Monroe *et al.*, *Amer. Clin. Prod. Rev.*, *5*:34-41 (1986)).

[0058] The particular label or detectable group used in the assay is not a critical aspect of the invention, as long as it does not significantly interfere with the specific binding of the antibody used in the assay. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and, in general, most any label useful in such methods can be applied to the present invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include magnetic beads (*e.g.*, DYNABEADS^{TM}), fluorescent dyes (*e.g.*, fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels, enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic beads (*e.g.*, polystyrene, polypropylene, latex, etc.).

[0059] The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on sensitivity required, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

[0060] Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (*e.g.*, biotin) is covalently bound to the molecule. The ligand then binds to another molecule (*e.g.*, streptavidin), which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. The ligands and their targets can be used in any suitable combination with antibodies that recognize SARS-related polypeptide, or secondary antibodies that recognize anti-SARS-related polypeptide.

[0061] The molecules can also be conjugated directly to signal generating compounds, *e.g.*, by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidotases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, etc. Chemiluminescent compounds include luciferin, and 2,3-dihydrophthalazinediones, *e.g.*, luminol. For a review of various labeling or signal producing systems that may be used, see U.S. Patent No. 4,391,904.

[0062] Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence may be detected visually, by means of photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Finally simple colorimetric labels may be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

[0063] Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of the target antibody is detected by simple visual inspection.

D. CROSS-REACTIVITY DETERMINATIONS

[0064] Immunoassays in the competitive binding format can also be used for cross-reactivity determinations. For example, a peptide having the sequence of SEQ ID NO:7, SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:18, can be immobilized to a solid support. Proteins (*e.g.*, SARS-related polypeptides and related peptides, *e.g.*, coronavirus peptide homologs or Adelaide River virus peptide homologs) are added to the assay that compete for binding of the antisera to the immobilized antigen. The ability of the added proteins to compete for

binding of the antisera to the immobilized protein is compared to the ability of SARS-related peptide to compete with itself. The percent crossreactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10% crossreactivity with each of the added proteins listed above are selected and pooled. The cross-reacting antibodies are optionally removed from the pooled antisera by immunoabsorption with the added considered proteins, *e.g.*, distantly related homologs.

[0065] The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described above to compare a second protein, thought to be perhaps a variant of SARS-related peptide to the peptide of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5. In order to make this comparison, the two proteins are each assayed at a wide range of concentrations and the amount of each protein required to inhibit 50% of the binding of the antisera to the immobilized protein is determined. If the amount of the second protein required to inhibit 50% of binding is less than 10 times the amount of the SARS-related peptide of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5 that is required to inhibit 50% of binding, then the second protein is said to specifically bind to the polyclonal antibodies generated to the SARS-related peptide immunogen.

E. DIAGNOSIS OR DETECTION OF SARS

[0066] One of skill in the art understands that the immunoassays described above can also be used to detect the presence of antibodies to SARS-related peptide in serum from a patient known to, or suspected of, having SARS. Such assays can be used as a criterion in diagnosing SARS or the susceptibility to SARS.

[0067] SARS-related peptide can also be used as a reagent to evaluate the efficacy of a vaccine. For example, a patient who is injected with a vaccine for the SARS infectious agent can be tested for the presence of antibodies to the infectious agent using SARS-related peptide.

[0068] In certain embodiments, *e.g.*, diagnosis of a current or past SARS infection, the level of SARS-related peptide, or antibodies to SARS-related peptide, are quantified. In such embodiments, the difference between the level of SARS-related peptide, or antibody to SARS-related peptides in a biological sample from a patient having, or suspected of having SARS is determined. Typically, a diagnostic presence often represents at least about a 10% increase, typically a 25%, 50%, 75%, or 100% increase, over a background level in the biological sample compared to a level expected in a control sample, such as a

sample of biological material representative of a healthy subject or normal tissue. Detection of SARS-related peptide or antibody to SARS-related peptide can be performed *in vitro, i.e.*, in cells within a biological sample taken from the mammal, or *in vivo*.

[0069] In addition to the immunoassays described above, SELDI can be used to detect the the SARS-related polypeptides of the present invention. SELDI involves the capture of analyte molecules on a solid support, such as a mass spectrometry probe surface, derivatized with an adsorbent that captures target analytes from a mixture. Typically a matrix material is applied to the captured analytes and the analytes are detected by laser desorption mass spectrometry. The adsorbents used can be chromatographic or biospecific. (*See, e.g.*, U.S. Patent No. 5,719,060 (Hutchens and Yip) and U.S. Patent No. 6,225,047 (Hutchens and Yip)). SELDI-based biochips and instruments are available from Ciphergen Biosystems, Inc., Fremont, CA.

[0070] In the present case, SARS-related polypeptides can be detected on both chromatographic and biospecific SELDI biochips. Typically, the sample is applied to the chip and allowed to incubate to promote adsorption. Then the biochip is washed and SARS-related polypeptides are detected by mass spectrometry.

[0071] Alternatively, the SARS-related polypeptides can be detected on SELDI biochips having SARS-related antibodies attached to their surface. In one embodiment, a SELDI biochip comprising functional groups such as carboxodiimizole or epoxide (*e.g.*, a PS10 or a PS20 ProteinChip array, Ciphergen Biosystems, Inc.) is derivatized with the antibodies by incubating the antibodies on the biochip surface in a reaction buffer. Then the sample is applied to the chip surface, incubated to promote binding, and washed. Proteins are detected by mass spectrometry.

[0072] This invention also provides polynucleotide probes having nucleotide sequences encoding SEQ. ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5, or their complementary strands. Such probes are useful in cloning the full 45 KD protein and in identifying nucleic acids encoding SARS-related polypeptides in a sample. As is well known in the art, there are many nucleotide sequences that can encode these SAR-related polypepdides, due to the degeneracy of the genetic code. However, the identification of such nucleotide sequences is well within the ability of a skilled artisan.

[0073] In addition to the foregoing, it has now been determined that naturally occurring defensins are present in decreased levels in individual having a viral infection associated with SARS compared to naturally occurring defensins levels present in healthy, non-infected individuals. As such, in one embodiment, the present invention provides a

method of diagnosing a viral infection associated with SARS in a human having at least one symptom associated with SARS, the method comprising: detecting decreased levels of defensin in a biological sample relative to normal, thereby diagnosing viral infection associated with SARS. Accordingly, decreased defensin levels relative to normal are indicative of viral infection associated with SARS. "Normal," as used herein, refers to defensin levels found in a comparable sample from a healthy, non-infected individual. Typically, a diagnostic presence often represents at least about a 10% decrease, typically a 25%, 50%, 75%, or 100% decrease compared to defensin levels expected in a control sample, such as a sample of biological material representative of a healthy, non-infected subject or normal tissue.

[0074] In a preferred embodiment, decreased levels of alpha-defensin polypeptides or alpha-defensin mRNA are detected. In a particularly preferred embodiment, decreased levels of alpha-defensin 1, 2, 3 or combinations thereof are detected.

[0075] Examples of symptoms associated with SARS include, but are not limited, fever, myalgias, headache, sore throat, dry cough, shortness of breath or difficulty breathing, *etc.* In some embodiments, the individual will have more than one symptom associated with SARS (*e.g.*, fever and dry cough, fever and difficulty breathing, *etc.*).

[0076] In another embodiment, the present invention provides a method of diagnosing a viral infection associated with SARS in a human who has traveled to a country having an outbreak of SARS or who has been in contacted with an individual who has or who is thought to have SARS, the method comprising: detecting decreased levels of defensin in a biological sample relative to normal, thereby diagnosing viral infection associated with SARS. In some embodiments, the individual will have at least one symptom associated with SARS as described above.

[0077] As explained herein, defensin levels can be measured using standard techniques know to and used by those of skill in the art. Therefore, methods known to those of skill in the art for detection of nucleic acids and proteins can be used for diagnosis and prognosis of viral infection associated with SARS, *e.g.*, PCR, northern and Southern blots, reverse transcription and amplification of mRNA, isolation of total RNA or poly A^+ RNA , dot blots, nucleic acid arrays, western blots, *in-situ* hybridization, immunoassays such as immunoprecipitation, ELISA, proteomics assays, polynucleotide array technology *and the like.*

[0078] In a preferred embodiment, immunological binding assays are used to detect defensin levels. Immunological binding assays (or immunoassays) typically use an

antibody that specifically binds to a protein or antigen of choice (in this case the defensin protein or antigenic subsequence thereof). The antibody (*e.g.*, anti-defensin) may be produced by any of a number of means well known to those of skill in the art. Additionally, antibodies capable of specifically binding to defensin polypeptides are commercially available and include, for example, the anti-HNP 1-3 antibody which is commercially available from HyCult Biotechnology, The Netherlands). Detection of defensin polypeptides using this antibody can be carried out using the methodology set forth in Example 3.

F. METHODS OF INHIBITING VIRAL INFECTION AND REPLICATION – "THERAPIES FOR TREATING SARS"

[0079] As described herein, defensin proteins, nucleic acids encoding defensin proteins, and small organic molecules, or fragments thereof, that increase defensin activity or expression can be used to inhibit viral infection associated with SARS, *e.g.*, by killing virally infected cells or by inhibiting viral replication. Defensins can be used therapeutically or prophylactically in a person. For instance, the defensin proteins, nucleic acids encoding defensin proteins, and small organic molecules can also be used prophylactically, *e.g.*, after exposure or suspected exposure to SARS to prevent infection or to kill infected cells.

[0080] Methods known in the art for therapeutic delivery of nucleic acids and proteins to an individual can be used in the methods of the present invention for treating or preventing viral infection associated with SARS in a subject, *e.g.*, cellular transfection, gene therapy, direct administration with a delivery vehicle or pharmaceutically acceptable carrier, indirect delivery by providing recombinant cells or regulatory sequences that enhance production of endogenous protein.

1. Cellular Transfection and Gene Therapy

[0081] The present invention provides the nucleic acid sequences of the defensin proteins which can be used for the transfection of cells *in vitro* and *in vivo*. These nucleic acids can be inserted into any of a number of well-known vectors for the transfection of target cells and organisms as described below. The nucleic acids are transfected into cells, *ex vivo* or *in vivo*, through the interaction of the vector and the target cell. The nucleic acid, under the control of a promoter, then expresses a defensin protein of the present invention, thereby mitigating the effects of low, absent, partial inactivation, or abnormal expression of a defensin gene, particularly as it relates to viral infection associated with SARS. The compositions are administered (*e.g.*, by injection into a muscle) to a patient in an amount

sufficient to elicit a therapeutic response in the patient. An amount adequate to accomplish this is defined as "therapeutically effective dose or amount."

[0082] In another aspect, the present invention provides a method of inhibiting viral infection associated with SARS in a human comprising transfecting a cell with a nucleic acid comprising a nucleotide sequence encoding a defensin polypeptide wherein the nucleic acid comprises an inducible promoter operably linked to the nucleotide sequence encoding the defensin. In one embodiments, expression of defensin proteins from eukaryotic vectors can be regulated using inducible promoters. With inducible promoters, expression levels are tied to the concentration of inducing agents, such as tetracycline or ecdysone, by the incorporation of response elements for these agents into the promoter. Generally, high level expression is obtained from inducible promoters only in the presence of the inducing agent; basal expression levels are minimal. Inducible expression vectors are often chosen if expression of the protein of interest is detrimental to eukaryotic cells. Other regulatory structures, *e.g.*, enhancers, can be used to increase expression of the defensin polypeptide.

[0083] Such gene therapy procedures have been used to correct acquired and inherited genetic defects, cancer, and other diseases in a number of contexts. The ability to express artificial genes in humans facilitates the prevention and/or cure of many important human diseases, including many diseases which are not amenable to treatment by other therapies (for a review of gene therapy procedures, *see* Anderson, *Science, 256*:808-813 (1992); Nabel *et al.*, *TIBTECH, 11*:211-217 (1993); Mitani *et al.*, *TIBTECH, 11*:162-166 (1993); Mulligan, *Science, 926*-932 (1993); Dillon, *TIBTECH, 11*:167-175 (1993); Miller, *Nature, 357*:455-460 (1992); Van Brunt, *Biotechnology, 6(10)*:1149-1154 (1998); Vigne, *Restorative Neurology and Neuroscience, 8*:35-36 (1995); Kremer *et al.*, *British Medical Bulletin, 51(1)*:31-44 (1995); Haddada *et al.*, in *Current Topics in Microbiology and Immunology* (Doerfler & Böhm eds., 1995); and Yu *et al.*, *Gene Therapy, 1*:13-26 (1994)).

[0084] In another aspect of the present invention, defensin nucleic acids are not introduced into a cell by transfection, but instead an exogenous regulator sequence, *e.g.*, promoter or enhancer, an exogenous exon, either coding or noncoding, and a splice donor site are introduced into a preselected site in the genome for homologous recombination with a defensin gene. Using these methods, exogenously supplied expression sequences recombine with genomic DNA allowing defensins to be produced in human cells using the naturally-occurring endogenous exons encoding these proteins. Such methods are described in U.S. Patent No. 5,733,746, which issued to Treco *et al.*

2. *Pharmaceutical Compositions and Administration*

[0085] Pharmaceutically acceptable carriers are determined in part by the particular composition being administered (*e.g.*, nucleic acid, protein, modulatory compounds such as small organic molecules or transduced cell), as well as by the particular method used to administer the composition. The present invention encompasses delivery of pharmaceutical compositions comprising proteins, nucleic acids, and small organic molecules, and the like. Accordingly, there are a wide variety of suitable formulations of pharmaceutical compositions of the present invention (*see, e.g., Remington's Pharmaceutical Sciences*, 17th ed., 1989). Administration can be in any convenient manner, *e.g.*, by injection, oral administration, inhalation, transdermal application, or rectal administration.

[0086] Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the packaged nucleic acid suspended in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, *e.g.*, sucrose, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art.

[0087] The compound of choice, alone or in combination with other suitable components, can be made into aerosol formulations (*i.e.*, they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

[0088] Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be

administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. Parenteral administration and intravenous administration are the preferred methods of administration. The formulations of commends can be presented in unit- or multi-dose sealed containers, such as ampules and vials.

[0089] Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Cells transduced by nucleic acids for *ex vivo* therapy can also be administered intravenously or parenterally as described above.

[0090] An important factor in the administration of polypeptide compounds is ensuring that the polypeptide has the ability to traverse the plasma membrane of a cell, or the membrane of an intra-cellular compartment such as the nucleus. Cellular membranes are composed of lipid-protein bilayers that are freely permeable to small, nonionic lipophilic compounds and are inherently impermeable to polar compounds, macromolecules, and therapeutic or diagnostic agents. However, proteins and other compounds such as liposomes have been described, which have the ability to translocate polypeptides across a cell membrane.

[0091] In addition, it can be important to alter the properties of the polypeptide, so that it is less "sticky" and does not form aggregates or bind to other proteins, or so that it has enhanced pharmacokinetics (*e.g.*, a longer half life) and is more stable, *e.g.*, in serum or while being stored, *e.g.*, by PEGylating the protein or conjugating the protein with a lipid or other moiety. It may also be important to provide a signal peptide, leader sequence, or a secretory peptide to the protein, for secretion *in vivo* or *in vitro*. Defensin polypeptides of the invention can also be specifically targeted to cells using a targeting moiety, such as a ligand that binds to a cell surface molecule, *e.g.*, antibodies, ligands, receptors, etc. Accordingly, the present invention provides delivery vehicles for the defensins described herein, as well as fusion proteins that have a heterologous moiety that has the ability to stabilize, specifically deliver or target, secrete, provide a detectable label, etc. for the defensin protein. In one embodiment, the fusion protein comprises an albumin moiety and a defensin moiety. The albumin moiety can be at least a portion of albumin (*e.g.*, human albumin) sufficient to extend the life of the defensin. Such constructs are described in more detail in International PCT Publication No. WO 01/79480, October 25, 2001 (Rosen *et al.*, "Albumin Fusion Proteins").

[0092] For example, "membrane translocation polypeptides" have amphiphilic or hydrophobic amino acid subsequences that have the ability to act as membrane-

translocating carriers. In one embodiment, homeodomain proteins have the ability to translocate across cell membranes. The shortest internalizable peptide of a homeodomain protein, Antennapedia, was found to be the third helix of the protein, from amino acid position 43 to 58 (*see, e.g.*, Prochiantz, *Current Opinion in Neurobiology*, *6*:629-634 (1996)). Another subsequence, the h (hydrophobic) domain of signal peptides, was found to have similar cell membrane translocation characteristics (*see, e.g.*, Lin *et al., J. Biol. Chem., 270(1)*:4255-14258 (1995)).

[0093] Examples of peptide sequences include, but are not limited to an 11 amino acid peptide of the tat protein of HIV; a 20 residue peptide sequence which corresponds to amino acids 84-103 of the p16 protein (*see* Fahraeus *et al., Current Biology, 6*:84 (1996)); the third helix of the 60-amino acid long homeodomain of Antennapedia (Derossi *et al., J. Biol. Chem., 269*:10444 (1994)); the h region of a signal peptide such as the Kaposi fibroblast growth factor (K-FGF) h region (Lin *et al., supra*); or the VP22 translocation domain from HSV (Elliot *et al., Cell, 88*:223-233 (1997)). Other suitable chemical moieties that provide enhanced cellular uptake can also be chemically linked to the defensins of the invention.

[0094] Toxin molecules also have the ability to transport polypeptides across cell membranes. Often, such molecules (called "binary toxins") are composed of at least two parts: (1) a translocation or binding domain or polypeptide, and (2) a separate toxin domain or polypeptide. Typically, the translocation domain or polypeptide binds to a cellular receptor, and then the toxin is transported into the cell. Several bacterial toxins, including *Clostridium perfringens* iota toxin, diphtheria toxin (DT), *Pseudomonas* exotoxin A (PE), pertussis toxin (PT), *Bacillus anthracis* toxin, and pertussis adenylate cyclase (CYA), have been used in attempts to deliver peptides to the cell cytosol as internal or amino-terminal fusions (Arora *et al., J. Biol. Chem., 268*:3334-3341 (1993); Perelle *et al., Infect. Immun., 61*:5147-5156 (1993); Stenmark *et al., J. Cell Biol., 113*:1025-1032 (1991); Donnelly *et al., PNAS, 90*:3530-3534 (1993); Carbonetti *et al., Abstr. Annu. Meet. Am. Soc. Microbiol., 95*:295 (1995); Sebo *et al., Infect. Immun., 63*:3851-3857 (1995); Klimpel *et al., PNAS U.S.A., 89*:10277-10281 (1992); Novak *et al., J. Biol. Chem., 267*:17186-17193 1992), and U.S. Patent Nos. 5,602,095, 4,892,827, and 5,608,039).

[0095] Such subsequences can be used to translocate defensins across a cell membrane. Defensins can be conveniently fused to or derivatized with such sequences. Typically, the translocation sequence is provided as part of a fusion protein. Optionally, a

linker can be used to link the defensin and the translocation sequence. Any suitable linker can be used, *e.g.*, a peptide linker or other chemical linkers.

[0096] The defensin can also be introduced into an animal cell, preferably a mammalian cell, via a microparticles and liposomes and liposome derivatives such as immunoliposomes. The term "liposome" refers to vesicles comprised of one or more concentrically ordered lipid bilayers, which encapsulate an aqueous phase. The aqueous phase typically contains the compound to be delivered to the cell.

[0097] The liposome fuses with the plasma membrane, thereby releasing the drug into the cytosol. Alternatively, the liposome is phagocytosed or taken up by the cell in a transport vesicle. Once in the endosome or phagosome, the liposome either degrades or fuses with the membrane of the transport vesicle and releases its contents.

[0098] In current methods of drug delivery via liposomes, the liposome ultimately becomes permeable and releases the encapsulated compound at the target tissue or cell. For systemic or tissue specific delivery, this can be accomplished, for example, in a passive manner wherein the liposome bilayer degrades over time through the action of various agents in the body. Alternatively, active drug release involves using an agent to induce a permeability change in the liposome vesicle. Liposome membranes can be constructed so that they become destabilized when the environment becomes acidic near the liposome membrane (*see, e.g., PNAS, 84*:7851 (1987); *Biochemistry, 28*:908 (1989)). When liposomes are endocytosed by a target cell, for example, they become destabilized and release their contents. This destabilization is termed fusogenesis. Dioleoylphosphatidylethanolamine (DOPE) is the basis of many "fusogenic" systems.

[0099] Such liposomes typically comprise a defensin of choice and a lipid component, *e.g.*, a neutral and/or cationic lipid, optionally including a receptor-recognition molecule such as an antibody that binds to a predetermined cell surface receptor or ligand (*e.g.*, an antigen). A variety of methods are available for preparing liposomes as described in, *e.g.*, Szoka *et al., Ann. Rev. Biophys. Bioeng., 9*:467 (1980), U.S. Patent Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, 4,946,787, PCT Publication No. WO 91\17424, Deamer *et al., Biochem. Biophys. Acta, 443*:629-634 (1976); Fraley, *et al., PNAS, 76*:3348-3352 (1979); Hope *et al., Biochem. Biophys. Acta, 812*:55-65 (1985); Mayer *et al., Biochem. Biophys. Acta, 858*:161-168 (1986); Williams *et al., PNAS, 85*:242-246 (1988); *Liposomes* (Ostro (ed.), 1983, Chapter 1); Hope *et al., Chem. Phys. Lip., 40*:89 (1986); Gregoriadis, *Liposome Technology* (1984) and Lasic, *Liposomes: from Physics to*

Applications (1993)). Suitable methods include, for example, sonication, extrusion, high pressure/homogenization, microfluidization, detergent dialysis, calcium-induced fusion of small liposome vesicles and ether-fusion methods, all of which are well known in the art.

[0100] In certain embodiments of the present invention, it is desirable to target the liposomes of the invention using targeting moieties that are specific to a particular cell type, tissue, and the like. Targeting of liposomes using a variety of targeting moieties (*e.g.*, ligands, receptors, and monoclonal antibodies) has been previously described (*see, e.g.*, U.S. Patent Nos. 4,957,773 and 4,603,044).

[0101] Standard methods for coupling targeting agents to liposomes can be used. These methods generally involve incorporation into liposomes lipid components, *e.g.*, phosphatidylethanolamine, which can be activated for attachment of targeting agents, or derivatized lipophilic compounds, such as lipid derivatized bleomycin. Antibody targeted liposomes can be constructed using, for instance, liposomes which incorporate protein A (*see,* Renneisen *et al.*, *J. Biol. Chem.*, *265*:16337-16342 (1990) and Leonetti *et al.*, *PNAS*, *87*:2448-2451 (1990).

[0102] In another embodiment, the defensin polypeptides of the present invention can be used as an adjuvant. More particularly, defensin polypeptides can be used as adjuvants to boost immunogenecity. As such, defensin polypeptides or nucleic acids encoding such defensin polypeptides can be used in combination with vaccines or other antigens to increase the antigenic response.

[0103] The methods of the present invention treat or prevent viral infection associated with SARS in a subject. The amount of defensin adequate to accomplish this is defined as a "therapeutically effective dose". Single or multiple administrations of defensins or defensin formulations can be administered depending on the dosage and frequency as required and tolerated by the patient. The formulations should provide a sufficient quantity of active agent, *i.e.*, defensin, to effectively treat or prevent viral infection associated with SARS in a subject.

[0104] The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the patient over time. The dose will be determined by the efficacy of the particular delivery method employed, the composition to be administered, such as nucleic acid, polypeptide, or small organic molecule, and the condition of the patient, as well as the body weight or surface area of the patient to be treated. The size of the dose also will be determined by the existence,

nature, and extent of any adverse side-effects that accompany the administration of a particular defensin composition, or transduced cell type in a particular patient.

[0105] For administration, compounds of the present invention can be administered at a rate determined by the LD-50 of the candidate compound, and the side-effects of the candidate compound at various concentrations, as applied to the mass and overall health of the patient. Administration can be accomplished via single, multiple, or divided doses. In general, the dosages will range from 1 microgram to 100 mg per kg of body weight, from 1 microgram to 1 mg per kg of body weight, or from 10 micrograms to 10 mg per kg of body weight. Those of skill would understand that other ranges may be suitable and could readily be ascertained. For example, a particular composition may be more effective at higher or lower doses. By evaluating a patient using the methods described herein, a skilled practitioner will be able to determine whether a patient is responding to treatment and will know how to adjust the dosage levels accordingly.

3. Combination Therapies

[0106] In numerous embodiments, the defensin polypeptides of the present invention may be administered in combination with one or more additional compounds or therapies. For example, multiple defensin polypeptides can be co-administered, or one or more defensin polypeptides can be administered in conjunction with one or more therapeutic compounds. In one embodiment, the other therapeutic agent is one that is used to prevent or treat viral infections. In another embodiment, the other therapeutic agent is an agent used to treat an opportunistic infection associated with viral infections.

G. SARS VACCINES: FORMULATIONS AND ADMINISTRATION

[0107] SARS vaccines can be formulated in accordance with standard techniques well known to those skilled in the pharmaceutical art. Typically, the vaccine is administered as a polypeptide, *e.g.*, a polypeptide comprising SEQ ID NO:7, although other vaccine compositions, *e.g.*, nucleic acid constructs encoding the polypeptide, may also be used. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical arts taking into consideration such factors as the age, sex, weight, and condition of the particular patient, and the route of administration.

[0108] The vaccines are administered to a patient in an amount sufficient to elicit a therapeutic effect, *i.e.*, an antibody response to the SARS polypeptide, or a cellular immune response including induction of $CD8^+$and/or $CD4^+$ cells. that prevents infection or at

least partially arrests or slows symptoms and/or complications of SARS infection. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend on, *e.g.*, the particular composition of the vaccine regimen administered, the manner of administration, the general state of health of the patient, and the judgment of the prescribing physician.

[0109] Generally the dosage for an initial prophylactic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg. Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. This is followed by boosting dosages of between about 1.0 μg to about 50,000 μg of peptide administered at defined intervals, typically from about four weeks to six months after the initial administration of vaccine. The immunogenicity of the vaccine can be assessed by measuring the level of antibodies in the blood.

[0110] The concentration of peptides of the invention in the pharmaceutical formulations can vary widely, *i.e.*, from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

[0111] A human unit dose form of the peptide composition is typically included in a pharmaceutical composition that comprises a human unit dose of an acceptable carrier, preferably an aqueous carrier, and is administered in a volume of fluid that is known by those of skill in the art to be used for administration of such compositions to humans (*see*, *e.g.*, Remington's Pharmaceutical Sciences, 17th Edition, A. Gennaro, Editor, Mack Publising Co., Easton, Pennsylvania, 1985).

[0112] A SARS vaccine comprising a polypeptide of the invention can also be administered as a nucleic acid. "Nucleic acid-based vaccines" include both naked DNA and vectored DNA (within a viral capsid) where the nucleic acid encodes a SARS polypeptide that provides an immunoprotective response in the person being vaccinated. Nucleic acid vaccines are administered by methods well known in the art as described in Donnelly *et al.* (*Ann. Rev. Immunol.*, *15*:617-648 (1997)); Felgner *et al.* (U.S. Patent No. 5,580,859, issued December 3, 1996); Felgner (U.S. Patent No. 5,703,055, issued December 30, 1997); and Carson *et al.* (U.S. Patent No. 5,679,647, issued October 21, 1997), each of which is incorporated herein by reference. One skilled in the art would know that the choice of a

pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration of the nucleic acid vaccine.

[0113] For example, naked DNA or polynucleotide in an aqueous carrier can be injected into tissue, such as muscle, in amounts of from 10 µl per site to about 1 ml per site. The concentration of polynucleotide in the formulation is from about 0.1 µg/ml to about 20 mg/ml.

[0114] Examples of vaccine compositions of use for the invention include liquid preparations, for orifice, *e.g.*, oral, nasal, anal, vaginal, etc. administration, such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (*e.g.*, injectable administration) such as sterile suspensions or emulsions. In such compositions the SARS polypeptide or vector encoding the polypeptide can be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like.

[0115] In one embodiment, the vaccines can also be formulated for administration via the nasal passages. Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of about 10 to about 500 microns which is administered in the manner in which snuff is taken, *i.e.*, by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid for administration as, for example, nasal spray, nasal drops, or by aerosol administration by nebulizer, include aqueous or oily solutions of the active ingredient. For further discussions of nasal administration of vaccines, references are made to the following patents, U.S. 5,846,978, 5,663,169, 5,578,597, 5,502,060, 5,476,874, 5,413,999, 5,308,854, 5,192,668, and 5,187,074.

[0116] The vaccines can be incorporated, if desired, into liposomes, microspheres or other polymer matrices (*see, e.g.*, Felgner *et al.*, U.S. Patent No. 5,703,055; Gregoriadis, *Liposome Technology*, Vols. I to III (2nd ed. 1993). Liposomes, for example, which consist of phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like.

[0117] Liposomes for use in the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a

sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, *e.g.*, liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, *e.g.*, Szoka *et al., Ann. Rev. Biophys. Bioeng.*, *9*:467 (1980), U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

[0118] Where susceptible individuals are identified prior to or during infection, the composition can be targeted to them, thus minimizing the need for administration to a larger population.

[0119] The vaccine may be delivered in a physiologically compatible solution such as sterile PBS in a volume of, *e.g.*, one ml. The vaccines may also be lyophilized prior to delivery. A variety of aqueous carriers may be used in administering the composition, *e.g.*, water, buffered water, 0.8% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservatives, and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

[0120] For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, and more preferably at a concentration of 25%-75%.

[0121] For aerosol administration, the immunogenic peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of peptides are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides

may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, *e.g.*, lecithin for intranasal delivery.

[0122] The compositions included in the vaccine regimen can be administered alone, or can be co-administered or sequentially administered with other immunological, antigenic, vaccine, or therapeutic SARS compositions. These include adjuvants, and chemical or biological agents given in combination with, or recombinantly fused to, a SARS antigen to enhance immunogenicity of the antigen. The co-administered or sequentially administered compositions can also include purified antigens from the SARS virus or nucleic acid expression cassettes that express the additional SARS antigen(s). The expression cassettes can be included on the same nucleic acid molecule that encodes the SARS polypeptide of the invention or can be encoded by a second recombinant molecule.

[0123] Examples of adjuvants which also may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). Again, co-administration is performed by taking into consideration such known factors as the age, sex, weight, and condition of the particular patient, and, the route of administration.

[0124] The vaccines can additionally be complexed with other components such as peptides, polypeptides and carbohydrates for delivery. For example, expression vectors, *i.e.*, nucleic acid vectors that are not contained within a viral particle, can be complexed to particles or beads that can be administered to an individual, for example, using a vaccine gun.

H. KITS

[0125] The invention also provides kits for carrying out the assays described herein. For example, such kits can comprise any one or more of the following materials: a SARS-related polypeptide, typically a synthetic SARS-related polypeptide; one or more antibodies specific to a SARS-related polypeptide; a detection label; and instructions for using the kit. A wide variety of kits and components can be prepared according to the present invention, depending upon the intended user of the kit and the particular needs of the user.

I. EXAMPLES

Example 1

[0126] The sequences were obtained as follows. Corona virus from patients diagnosed with SARS was grown in cell culture. The cells were lysed and a pellet was isolated by centrifugation. Proteins in the pellet were separated by SDS-PAGE and a western blot was performed using sera from SARS patients. Immunoglobulins from SARS patients bound to a protein having an apparent molecular weight of 49 kD. However, immunoglobulin from non-SARS patients did not bind to this protein.

[0127] The 49 kD protein was further purified using sucrose gradient centrifugation followed by SDS-PAGE. The intact protein then was detected by mass spectrometry and had an apparent molecular weight of about 45 kD.

[0128] A plug of the gel containing the 45 kD protein was destained and digested in-gel with trypsin. The resulting peptides were eluted and analyzed by qQ-TOF on a Q-Star (ABI/Sciex) fitted with a ProteinChip interface using a SEND ID biochip or an NP-20 ProteinChip array from Ciphergen Biosystems, Inc. using cyano hydroxyl cinammic acid.

[0129] The peptides detected in this manner were submitted for analysis by the NCBI non-redundant database for viral taxa. This analysis provided no high probability (> 90%) matches in the database.

[0130] MS-MS was then performed on several of the peptides. MS-MS analysis provided sufficient coverage to determine the amino acid sequence *de novo* of five of the peptides, identified below as Peptides 1-5. Two of these peptides have been sequenced and subjected to database mining analysis. Subsequent comparison of these sequences against the presumptive amino acid sequence of SARS protein of SEQ ID NO: 7 showed exact matches with Peptides 1-5.

[0131] Peptide 1 had an isotopic MH+ of 1183.5854 and has the amino acid sequence: QYNVTQAFGR (SEQ ID NO:1). In a test against a viral genome database the peptide exhibited 80% sequence homology with a single peptide from the spike protein of Canine Coronavirus.

[0132] Peptide 2 had an isotopic MH+ of 1851.8315* and comprises the sequence STDNNQNGG (SEQ ID NO:2).

[0133] Therefore, both the above two peptides have homology with viral envelope proteins whose function is host attachment.

[0134] Peptide 3 is 2151.0250 m/z comprising the C-terminal sequence NSGPDDQIGYYR (SEQ ID NO:3). Blast search shows the homology with S1 glycoprotein

from Avian infectious bronchitis virus for QIGYY (SEQ ID NO:20) and also homology with nucleocapsid proteins from many viruses (DDQIGY (SEQ ID NO:21)).

[0135] Peptide 4 is 886.4058 m/z. Comprises the C-terminal sequence EGSR (SEQ ID NO:4) . Blast search does not show any significant homology probably because the fragment is very short.

[0136] Peptide 5 is 1892.8617 m/z. We determined by *de novo* sequencing that this peptide included the sequence MDDFSR (SEQ ID NO:5).

[0137] The amino acid sequence of an immunogenic SARS polypeptide comprising the peptide fragments identified herein was determined from the SARS genomic sequence data. The amino acid sequence is shown in SEQ ID NO:7. The nucleic acid encoding the polypeptide is provided in SEQ ID NO:8.

Example 2

[0138] We performed an analysis *in silico* of the genomic sequence of the SARS virus released by the Genome Sciences Centre at the British Columbia Cancer Agency:

http://ybweb.bcgsc.ca/sars/TOR2_draft_genome_assembly_120403.fasta.

This involved *in silico* translation of the DNA sequence into amino acid sequences, followed by *in silico* tryptic digestion of the encoded amino acid sequences. The presumptive mass for each of the tryptic digestion fragments was then determined. The masses of twelve of the fragments determined by mass spectrometry in Example 1 matched the presumptive masses of the *in silico* tryptic digestion fragments in reading frame 1. The twelve matching peptide fragments are as follows:

PEPTIDE 1410.7748

1 Solid MSMS Match for Peptide KKTDEAQPLPQR (SEQ ID NO:9)

Amino Acids 3754-386 of SEQ ID NO: 7

1282m/z is a fragment of this peptide / significant homology between MSMS spectra of both

Largest ion at 938.51 matches c-terminal cleavage between D/E

PEPTIDE 1282.6856

1 Solid MSMS Match for Peptide KTDEAQPLPQR (Subset of 1410) (SEQ ID NO:10)

Amino Acids 376-386

938.51 again largest fragment matches c-terminal cleavage between D/E

PEPTIDE 1183.5854 (Peptide 1 of Example 1)

1 Solid MSMS Match for Peptide QYNVTQAFGR (SEQ ID NO:1)

Amino Acids 268-277

16/20 B & Y-Ions Matched

PEPTIDE 1774.8569

Very weak MSMS Data, but still significant matches

Matches Peptide GPEQTQGNFGDQDLIR (SEQ ID NO:11)

Amino Acids 279-294

Although the spectrum is weak, the majority of unmatched ions are weakly present

The two major fragments occur at c-terminal cleavages of D/Q and D/L

PEPTIDE 1851.8315* (Peptide 2)

Closer examination of the spectrum shows this peptide is de-amidated. The actual mass should be 1850.7809. Many of the fragments also reflect this shift.

Matches Peptide ITFGGPTDSTDNNQNGGR (SEQ ID NO:12)

Amino Acids 16-33

Two major fragments 759 / 1062 occur at c-terminal cleavages of D/N and D/S, respectively

PEPTIDE 1930.9335

Strong MSMS match to peptide RGPEQTQGNFGDQDLIR (SEQ ID NO:13)

1774m/z is a y-ion fragment of this peptide and there is significant homology between the two spectra

Amino acids 278-294

The two major fragments both match c-terminal fragmentation at D/Q

PEPTIDE 2151.0250 (Peptide 3)

Strong match to peptide GQGVPINTNSGPDDQIGYYR (SEQ ID NO:14)

Amino Acids 70-89

14/20 Y-ions matched

Two major fragments at 799 and 914 match c-terminal cleavage at D/Q and D/D, respectively

PEPTIDE 886.4058 (Peptide 4)

Strong match to peptide GFYAEGSR (SEQ ID NO:15)

Amino Acids 171-178

Lots of matrix chemical noise in the MSMS spectrum, all with .0 mass defect

Largest real fragment at 319 matches cleavage at E/G

PEPTIDE 2475.1607

1 Solid MSMS Match for Peptide ITFGGPTDSTDNNQNGGRNGARPK (SEQ ID NO:16)

This ion has 2 sites of de-amidatation

Amino Acids 16-39

1851m/z is a fragment of this peptide and is also deamidated

PEPTIDE 1892.8617 (Peptide 5)

1 Solid MSMS Match for Peptide QPTVTLLPAADMDDFSR (Oxidized Met) (SEQ ID NO:17)

Amino Acids 390-406

Extends the previous protein coverage toward c-terminus

PEPTIDE 2021.0617

1 Solid MSMS Match for Peptide KQPTVTLLPAADMDDFSR (SEQ ID NO:18)

Amino Acids 389-406

Only separated by 1 missed cleavage from 1892; n-terminal Lysine

Nearly identical spectrum to 1892

PEPTIDE 1145.4983

1 MS-MS Match for Peptide SDNGPQSNQ (SEQ ID NO:19)

N-terminus of 46kDa protein from reading frame 1 (n-terminal methionine lost, which is common)

Amino Acids 2-11

Thus, the mass characteristics and sequence characteristics of the protein from the SARS virus that we analyzed are consistent with a protein encoded by an open reading frame of the SARS viral genome. The nucleotide sequence of this open reading frame is provided in SEQ ID NO: 8 and the amino acid sequence encoded by the open reading frame is provided in SEQ ID NO: 7. The measured mass of the viral protein is about 45700. The predicted mass of the protein having the amino acid sequence of SEQ ID NO:7 is 46025. The protein has a predicted pI of 10.1. Thus, the measured mass is consistent with the mass of the polypeptide of SEQ ID NO:7 minus the initial methionine.

Example 3

[0139] This example demonstrates that patients having a viral infection associated with SARS have decreased defensin levels.

[0140] Nasal pharyngeal swabs were obtained from suspected SARS patients (all tested positive by Coronavirus gene PCR test) and from patients tested positive for metapneumovirus and other respiratory ailments (Health Canada). These were transferred to a carrier buffer containing bovine serum albumin and stored at –80°C until use.

[0141] 5 ul of nasal pharyngeal swabs in carrier were diluted with 10 µl of 9 M urea 2% CHAPS 50 mM HEPES pH7. After several minutes standing in ice, these were added to 125 µl of 0.25 M urea 0.1%CHAPS 50 mM sodium phosphate pH6 in a bioprocessor over a CM10 ProteinChip array (Ciphergen). After 30 min incubation at 4°C, the chips were washed with 125 µl of 0.25 M urea 0.1%CHAPS 50 mM sodium phosphate pH6 two times. After rinsing with water, the proteins retained on the chips were detected by SELDI with a PBSII (Ciphergen) in the presence of sinapinic acid (Ciphergen).

[0142] 1 ul of nasal pharyngeal swabs in carrier was diluted with 10 µl of 100 mM ammonium acetate 5% acetonitrile pH4. 5 µl was added to C18 SEND ProteinChip array. After 30 min incubation at 23°C, the samples were removed. 2 µl of 25% acetonitrile 0.1% trifluoroacetic acid were added. After air drying, the proteins retained on the chips were detected by SELDI with a PBSII (Ciphergen).

[0143] Immunoassay: An immunoassay was developed to detect defensin levels in both the SARS group and the metapneumovirus group. Defensin specific

ProteinChip® Arrays were prepared by covalently attaching anti-HNP 1-3 antibody (HyCult Biotechnology, The Netherlands) to discrete spots on carbonyldiimidazole-activated ProteinChip arrays. This was accomplished by adding 1ul of 0.39 mg/m1 antibody solution in sodium phosphate buffer (50 mM, pH 7.2) to each spot and incubating at room temperature and high humidity for 1 hr. After incubation, each spot was blocked by adding 5 µl of BSA (10 mg/ml) and incubating for an additional 1hr at room temperature and high humidity. Arrays were finally prepared by washing extensively in sodium phosphate buffer to remove any unbound material. One µl of nasopharyngeal swap fluid in transport buffer was diluted in 4 µl 50 mM sodium phosphate (pH 7.2) and added directly to each spot of the anti-defensin antibody array. After incubation for 1 hr at room temperature and high humidity, all spots on arrays were washed three times with 5 µl sodium phosphate buffer containing 1 M urea, 0.5 M NaCl and 0.1% triton-X100. Spots were then washed 2 times with 5 µl each wash HEPES buffer (5 mM, pH 7.2), then allowed to dry. 1 µl 20% CHCA in 50% MeCN and 0.1% TFA was then added and allowed to dry. The arrays were then analyzed using SELDI-TOF-MS detection. Figure 2 illustrates the results obtained on a Ciphergen CM10 chip. Figure 3 illustrates the results obtained on a Ciphergen C18 SEND chip. Figure 4 illustrates the results obtained using an SELDI immunoassay for detecting defensin levels. As illustrated in Figures 2, 3 and 4, defensin levels are decreased in SARS patients, *i.e.*, individuals having a viral infection associated with SARS.

[0144] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

J. SEQUENCE LISTING

SEQ ID NO:1 QYNVTQAFGR

SEQ ID NO:2 STDNNQNGG

SEQ ID NO:3 NSGPDDQIGYYR

SEQ ID NO:4 EGSR

SEQ ID NO:5 FDDYAR

SEQ ID NO:6: STDNNQ

SEQ ID NO:7: SARS polypeptide

MSDNGPQSNQRSAPRITFGGPTDSTDNNQNGGRNGARPKQRRPQGLPNNTASWFTA
LTQHGKEELRFPRGQGVPINTNSGPDDQIGYYRRATRRVRGGDGKMKELSPRWYFY
YLGTGPEASLPYGANKEGIVWVATEGALNTPKDHIGTRNPNNNAATVLQLPQGTTLP
KGFYAEGSRGGSQASSRSSSRSRGNSRNSTPGSSRGNSPARMASGGGETALALLLLD
RLNQLESKVSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKQYNVTQAFGRRGPE
QTQGNFGDQDLIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYHGAI
KLDDKDPQFKDNVILLNKHIDAYKTFPPTEPKKDKKKKTDEAQPLPQRQKKQPTVTL
LPAADMDDFSRQLQNSMSGASADSTQA*

SEQ ID NO:8: nucleic acid sequence encoding the SARS polypeptide

ATGTCTGATAATGGACCCCAATCAAACCAACGTAGTGCCCCCCGCATTACATTTG
GTGGACCCACAGATTCAACTGACAATAACCAGAATGGAGGACGCAATGGGGCAA
GGCCAAAACAGCGCCGACCCCAAGGTTTACCCAATAATACTGCGTCTTGGTTCAC
AGCTCTCACTCAGCATGGCAAGGAGGAACTTAGATTCCCTCGAGGCCAGGGCGT
TCCAATCAACACCAATAGTGGTCCAGATGACCAAATTGGCTACTACCGAAGAGC
TACCCGACGAGTTCGTGGTGGTGACGGCAAAATGAAAGAGCTCAGCCCCAGATG
GTACTTCTATTACCTAGGAACTGGCCCAGAAGCTTCACTTCCCTACGGCGCTAAC
AAAGAAGGCATCGTATGGGTTGCAACTGAGGGAGCCTTGAATACACCCAAAGAC

CACATTGGCACCCGCAATCCTAATAACAATGCTGCCACCGTGCTACAACTTCCTC
AAGGAACAACATTGCCAAAAGGCTTCTACGCAGAGGGAAGCAGAGGCGGCAGT
CAAGCCTCTTCTCGCTCCTCATCACGTAGTCGCGGTAATTCAAGAAATTCAACTC
CTGGCAGCAGTAGGGGAAATTCTCCTGCTCGAATGGCTAGCGGAGGTGGTGAAA
CTGCCCTCGCGCTATTGCTGCTAGACAGATTGAACCAGCTTGAGAGCAAAGTTTC
TGGTAAAGGCCAACAACAACAAGGCCAAACTGTCACTAAGAAATCTGCTGCTGA
GGCATCTAAAAAGCCTCGCCAAAAACGTACTGCCACAAAACAGTACAACGTCAC
TCAAGCATTTGGGAGACGTGGTCCAGAACAAACCCAAGGAAATTTCGGGGACCA
AGACCTAATCAGACAAGGAACTGATTACAAACATTGGCCGCAAATTGCACAATT
TGCTCCAAGTGCCTCTGCATTCTTTGGAATGTCACGCATTGGCATGGAAGTCACA
CCTTCGGGAACATGGCTGACTTATCATGGAGCCATTAAATTGGATGACAAAGATC
CACAATTCAAAGACAACGTCATACTGCTGAACAAGCACATTGACGCATACAAAA
CATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAAAAAGACTGATGAAGCTC
AGCCTTTGCCGCAGAGACAAAAGAAGCAGCCCACTGTGACTCTTCTTCCTGCGGC
TGACATGGATGATTTCTCCAGACAACTTCAAAATTCCATGAGTGGAGCTTCTGCT
GATTCAACTCAGGCATAA

SEQ ID NO:9 KKTDEAQPLPQR

SEQ ID NO:10 KTDEAQPLPQR

SEQ ID NO:11 GPEQTQGNFGDQDLIR

SEQ ID NO:12 ITFGGPTDSTDNNQNGGR

SEQ ID NO:13 RGPEQTQGNFGDQDLIR

SEQ ID NO:14 GQGVPINTNSGPDDQIGYYR

SEQ ID NO:15 GFYAEGSR

SEQ ID NO:16 ITFGGPTDSTDNNQNGGRNGARPK

SEQ ID NO:17 QPTVTLLPAADMDDFSR

SEQ ID NO:18 KQPTVTLLPAADMDDFSR

SEQ ID NO:19 SDNGPQSNQ

SEQ ID NO:20 QIGYY

SEQ ID NO:21 DDQIGY

SEQ ID NO:22 The 46kDa protein translated from the SARS genome

M***SDNGPQSNQ***RSAPR***ITFGGPTDSTDNNQNGGRNGARPK***QRRPQGLPNNTASWFTA
LTQHGKEELRFPR***GQGVPINTNSGPDDQIGYYR***RATRRVRGGDGKMKELSPRWYFY
YLGTGPEASLPYGANKEGIVWVATEGALNTPKDHIGTRNPNNNAATVLQLPQGTTLP
K***GFYAEGSR***GGSQASSRSSSRSRGNSRNSTPGSSRGNSPARMASGGGETALALLLLD
RLNQLESKVSGKGQQQQGQTVTKKSAAEASKKPRQKRTATK***QYNVTQAFGRRGPE***
QTQGNFGDQDLIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYHGA
IKLDDKDPQFKDNVILLNKHIDAYKTFPPTEPKKDKK***KKTDEAQPLPQR***QK***KQPTVT***
LLPAADMDDFSRQLQNSMSGASADSTQA

WHAT IS CLAIMED IS:

1. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18.

2. The isolated polypeptide of claim 1, wherein said polypeptide is associated with Severe Acute Respiratory Syndrome (SARS).

3. An isolated polypeptide comprising the amino acid sequences of SEQ ID NO:1, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:18.

4. An isolated polypeptide having the amino acid sequence of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18.

5. The polypeptide of claim 4, wherein said polypeptide is associated with SARS.

6. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:7.

7. An isolated polypeptide having the amino acid sequence of SEQ ID NO:7.

8. An isolated nucleic acid encoding a polypeptide comprising SEQ ID NO:7

9.. An isolated nucleic acid of claim 8, wherein the nucleic acid comprises SEQ ID NO:8.

10. An isolated nucleic acid encoding a polypeptide having the amino acid sequence of SEQ ID NO:7.

11. An antibody that selectively binds to the polypeptide of claim 4 or claim 7.

12. A method of detecting the presence of an infection associated with Severe Acute Respiratory Syndrome (SARS) in a sample, said method comprising:

detecting the presence of a SARS-related polypeptide in said sample, wherein the presence of said polypeptide is indicative of the presence of said infection.

13. The method of claim 12, wherein said SARS-related polypeptide is a polypeptide of claim 1 or claim 6.

14. The method of claim 12, wherein said SARS-related polypeptide is a polypeptide of claim 4 or claim 7.

15. A method of detecting the presence of an infection associated with SARS in a sample, said method comprising:

detecting the presence of a SARS-related antibody in said sample, wherein the presence of said SARS antibody is indicative of the presence of said infection.

16. The method of claim 15, wherein said SARS-related antibody is an antibody of claim 11.

17. The method of claim 15, wherein said method is a western blot assay.

18. The method of claim 15, wherein said method is an enzyme linked immunoabsorbent assay (ELISA).

19. A kit for detecting antibodies indicative of infection associated with Severe Acute Respiratory Syndrome (SARS) in a mammal, said kit comprising a SARS-related polypeptide of claim 1, claim 4, claim 6, or claim 7.

20. The kit of claim 19, further comprising a reagent for detecting a complex comprising a polypeptide of claim 1, claim 4, claim 6, or claim 7 and at least one antibody specifically bound thereto.

21. The kit of claim 19, wherein said reagent is an anti-antibody.

22. The kit of claim 19, wherein said reagent for detecting said complex comprises a label.

23. The kit of claim 22, wherein said label is an enzyme.

24. A method of inducing an immune response to SARS virus, the method comprising administering a polypeptide of claim 6 or claim 7 to a patient at risk for SARS infection.

25. A method of treating a viral infection associated with Severe Acute Respiratory Syndrome in a mammal, the method comprising administering a defensin polypeptide to the mammal.

26. The method of claim 26, wherein the defensin polypeptide is an alpha-defensin polypeptide selected from the group consisting of alpha-defensin 1, alpha-defensin 2 and alpha-defensin 3.

27. The method of claim 26, further comprising administering a second alpha-defensin polypeptides selected from the group consisting of alpha-defensin 1, alpha-defensin 2 and alpha-defensin 3.

28. The method of claim 26, wherein the mammal is a human.

29. A method comprising administering a prophylactic amount of a defensin polypeptide to a person who is at high risk of viral infection associated with SARS.

30. The method of claim 29, wherein the defensin polypeptide is an alpha-defensin polypeptide selected from the group consisting of alpha-defensin 1, alpha-defensin 2 and alpha-defensin 3.

31. The method of claim 31, further comprising administering a second alpha-defensin polypeptide selected from the group consisting of alpha-defensin 1, alpha-defensin 2 and alpha-defensin 3.

32. The method of claim 31, wherein the alpha-defensin polypeptides are alpha-defensin 1, alpha-defensin 2 and alpha-defensin 3.

33. A method of inhibiting a viral infection associated with SARS in a human, the method comprising transfecting a cell with a nucleic acid comprising a nucleotide sequence encoding a defensin polypeptide or transfecting with a nucleic acid comprising a nucleotide sequence encoding a defensin polypeptide and administering the cell to the human.

34. The method of claim 33, wherein the defensin polypeptide is an alpha-defensin polypeptide selected from the group consisting of alpha-defensin 1, alpha-defensin 2 and alpha-defensin 3.

35. A method of diagnosing a viral infection associated with SARS in a human having at least one symptom associated with SARS, the method comprising: detecting decreased levels of defensin in a biological sample relative to normal, thereby diagnosing viral infection associated with SARS.

36. The method of claim 35, wherein the at least one symptom associated with SARS is a member selected from the group consisting of fever, myalgias, headache, sore throat, dry cough, shortness of breath and difficulty breathing.

37. The method of claim 35, wherein the defensin level is detected by measuring the amount of an alpha-defensin polypeptide present in the biological sample.

38 . The method of claim 37, wherein the alpha-defensin polypeptide is a member selected from the group consisting of alpha-defensin 1, alpha-defensin 2 and alpha-defensin 3.

39. The method of claim 35, wherein the defensin level is detected by measuring the amount of alpha-defensin mRNA present in the biological sample.

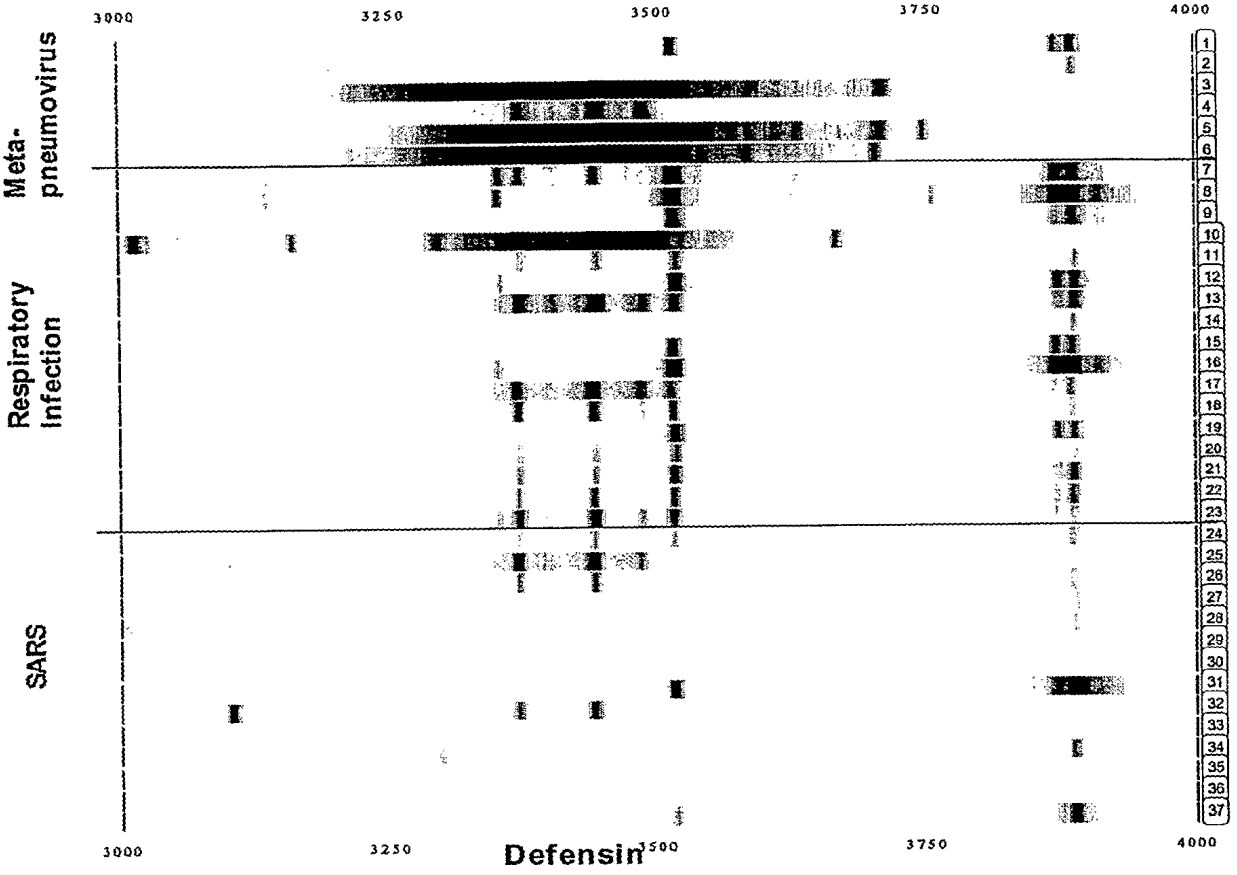
FIGURE 1

M***SDNGPQSNQ***RSAPR***ITFGGPTDSTDNNQNGGRNGARPK***QRRPQGLPNNTASW
FTALTQHGKEELRFPR***GQGVPINTNSGPDDQIGYYR***RATRRVRGGDGKMKELSPR
WYFYYLGTGPEASLPYGANKEGIVWVATEGALNTPKDHIGTRNPNNNAATVLQLPQ
GTTLPK***GFYAEGSR***GGSQASSRSSSRSRGNSRNSTPGSSRGNSPARMASGGGE
TALALLLLDRLNQLESKVSGKGQQQQGQTVTKKSAAEASKKPRQKRTATK***QYNVT***
QAFGRRGPEQTQGNFGDQDLIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVT
PSGTWLTYHGAIKLDDKDPQFKDNVILLNKHIDAYKTFPPTEPKKDKK***KKTDEAQPL***
PQRQK***KQPTVTLLPAADMDDFSR***QLQNSMSGASADSTQA

FIGURE 2


3000
3250
3500
3750
4000
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Defensin

FIGURE 3


3000
3250
3500
3750
4000
Meta-pneumovirus
Respiratory Infection
SARS
Defensin
1
2
3
4
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7
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FIGURE 4
Defensin SELDI Immunoassay